CAUCASIANA
Journal on the biodiversity of the Caucasus and the adjacent regions

New and well-forgotten: DNA-assisted records of two beetle (Insecta, Coleoptera) species new for the fauna of Georgia with an update on the distribution of some other little studied taxa

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http://zoobank.org/54DC89E9-2299-491E-915A-A7766F6CDCBC

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Academic editor: Alexander Prokin ♦ **Received:** 19 December 2022 ♦ **Accepted:** 8 March 2023 ♦ **Published:** 18 April 2023

Abstract

First records of two species of beetles (Insecta, Coleoptera), namely *Pocadius adustus* Reitter, 1888 (Nitidulidae) and *Byrrhus pustulatus* (Forster, 1770) (Byrrhidae) are given for Georgia. In addition, an update on the occurrences of *Necrobia violacea* (Linnaeus, 1758) (Cleridae), *Coelostoma orbiculare* (Fabricius, 1775) (Hydrophilidae) and *Lycoperdina succincta* (Linnaeus, 1767) (Endomychidae) are also presented based on the specimens collected in Samtskhe-Javakheti region, determined by morphological and mtDNA barcoding methods.

Key words

Biodiversity, CaBOL, fauna, South Caucasus

Introduction

Situated at the border of Europe and Asia, the Caucasus region represents one of the 36 biodiversity hotspots with an exceptional richness of endemic species and is, like the other biodiversity hotspots, critically prone to biodiversity loss (Myers et al. 2000; Habel et al. 2019; Mumladze et al. 2020). Surprisingly, particularly large gaps in biodiversity information exist in the countries of the Caucasus (Wetzel et al. 2018; Mumladze et al. 2020). The beetles, for instance, are exemplary in this respect (Aslan et al. 2017; Assing and Schülke 2017; Maghradze et al. 2019). Biodiversity is a key component and plays an extremely important role in ecosystem functioning and, thus ensuring sustainable life on our planet (Humphries et al. 1995). Therefore, knowledge of the Caucasus biodiversity is crucial from a conservation point of view, and a species inventory in Georgia may help to reduce the knowledge gaps and promote biodiversity conservation and sustainable development. The goal of the present study is to contribute new faunistic and distributional data on some little and understudied beetles of Georgia.

Materials and methods

Sampling

Material was collected by the CaBOL team (Caucasus Barcode of Life) (Thormann et al. 2019) of the Institute of Ecology, Ilia State University, during the CaBOL (https://

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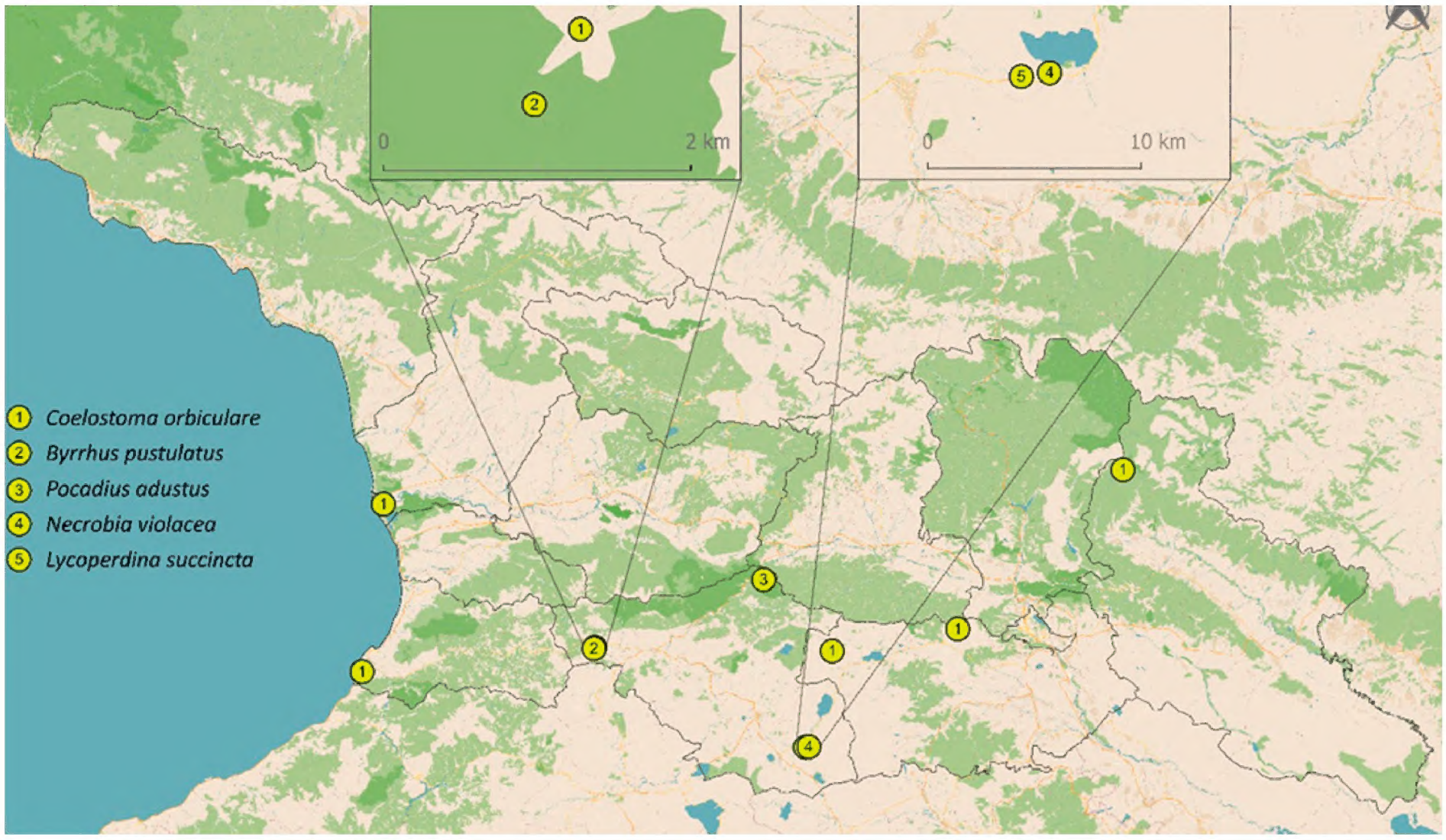

Figure 1. Map of the beetle sampling sites in Georgia.

ggbc.eu/) expeditions to the Samtskhe-Javakheti region, held on October 08-11, 2021 (Fig. 1). Hand collecting and aspirators were used for collecting beetles from under rocks, vegetation, wood bark, and mushrooms. The collected specimens were preserved in 96% ethanol and stored in a freezer at -22˚C at the scientific collections of Ilia State University (ISU). Additional material on *Coelostoma orbiculare* (Fabricius, 1775) was kindly provided by Levan Mumladze (Institute of Zoology, Ilia State University). All material has been determined by Shota Japarashvili and Armen Seropian.

Photos of preserved specimens were taken using a Canon EOS 550D camera with Canon EF 100mm f/2.8 Macro USM lens and Raynox DCR-250 Super Macro Snap-On Lens attached. Images of Pocadius adustus and Necrobia violacea were taken using a Canon EOS 60D camera with a Canon MP-E 65mm f/2.8 1–5x Macro Photo Lens mounted on a Novoflex Castel-L Focusing Rack. Digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6.

DNA processing

Genomic DNA was extracted from tissue samples using the Quick-DNATM Miniprep PlusKit (Zymo Research) (for 25 mg of tissue). Partial sequences of cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pairs LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95°C for 1 min, followed by the first cycle set (15 cycles): 94°C for 30 sec., annealing at 55°C for 1 min (–1°C per cycle) and extension at 72°C for 1:30 min. Second cycles set (25 cycles): 94°C for 35 sec., 45°C for 1 min, 72°C for 1:30 min, followed by 1 cycle at 72°C for 3 min and the final extension step at 72°C for 5 min. PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. Sequencing of the unpurified PCR products in both directions was conducted at the Beijing Genomics Institute (Hong Kong, CN) by using the amplification primers. Barcode sequences were uploaded to the BOLD Systems database (https://www.boldsystems.org/) (Ratnasingham and Hebert 2007) which was used for sequence identification and comparison. For each specimen/sequence studied, we provide the barcode index number (BIN) as defined by BOLD Systems along with CaBOL collection IDs. Extracted DNA was deposited in the scientific collections of Ilia State University, Tbilisi, Georgia.

Results

Order Coleopera Linnaeus, 1758

Family Hydrophilidae Latreille, 1802

Genus Coelostoma Brullé, 1835

Coelostoma orbiculare (Fabricius, 1775)

Materials Examined: GEORGIA • Ijareti; N41.6534°, E42.7048°; 1428 m a.s.l; 09.10.2021; ISU, CaBOL ID: 1018686. Poti; N42.1647°, E41.6896°; 1 m a.s.l.; 16.05.2021; ISU, CaBOL ID:1020485 (Fig. 2). East of village Mokhisi; N41.7188°, E44.4487°; 1635 m a.s.l.; 27.06.2021; ISU, CaBOL IDs: 1020505, 1020506, 1020507. North west of Ozni Village; N41.6400°, E43.8458°; 1961 m a.s.l.; 16.05.2017; ISU, CaBOL IDs: 1020131. Gonio; N41.5669°, E41.5669°; 2 m a.s.l.; 27.08.2019. Sakisto lake; N42.2871°, E45.2428°; 2250 m a.s.l.; 29.05.2022; ISU, CaBOL ID: 1033224. all leg: L. Mumladze.

Figure 2. *Coelostoma orbiculare* (Fabricius, 1775), Ijareti. A – dorsal view; B – ventral view. Scale bar = 1 mm.

Genetics: Four barcodes of the species were obtained from specimens with CaBOL-IDs 1020485, 1020505, 1020506, and 1020507 (BOLD: AAF3894). The newly obtained barcodes were nearly identical (maximum p-distance 0.05%), with the nearest neighbor in BOLD systems being *Coelostoma orbiculare* from Finland (BIN ID: AAF3894, with mean p-distance 0.57%). The morphology of the Georgian specimens also perfectly corresponds to the descriptions provided in the keys by Medvedev (1965), Shatrovsky (1992), and Jia et al. (2014).

Remarks: To date, 30 species of Hydrophilidae comprising 12 genera are known from Georgia (Przewoźny 2022; Tarkhnishvili et al. 2013). The water-scavenger beetle genus *Coelostoma* includes more than 100 described species, with *C. orbiculare* and *C. hispanicum* (Küster, 1848) being the only species of the genus in Europe (Przewoźny 2022). All species of *Coelostoma* for which biology is known, including *C. orbiculare*, are aquatic and collected either directly among submerged plants at the edge of water or from wet places along streams, rivers, and standing water (Shatrovsky 1992; Jia et al. 2014), where they may be found feeding on wet and submerged surfaces, including wet rocks and artificial concrete surfaces (Jia et al. 2014). The species has a wide distribution in the Palaearctic region, with the nearest records from Azerbaijan, Armenia, and Central-European Russia (Przewoźny 2022). The information on the occurrence of *C. orbiculare* in Tbilisi and Gomareti (Zaitsev 1953) was overlooked by subsequent authors, and since then, no study of the Hydrophilidae family in Georgia has been performed. Based on the material presented here, we can confirm that *C. orbiculare* has widespread distribution in Georgia (see Fig. 1).

Family Endomychidae Leach, 1815

Genus Lycoperdina Latreille, 1807

Lycoperdina succincta (Linnaeus, 1767)

Materials Examined: GEORGIA • Saghamo Lake; N41.2941°, E43.7309°; 2006 m a.s.l.; 11.04.2021; ISU, CaBOL ID: 1018736; leg: CaBOL team.

Genetics: The BOLD identification engine indicated high similarity of the sequence yielded from the specimen with CaBOL-ID 1018736 (Fig. 3) (BOLD: AEU7031) to the COI of *Lycoperdina succincta* from Germany (BIN ID: ACZ0793, similarity 97.69%). The morphology of the Georgian specimen also perfectly corresponds to the description provided in the keys by Kryzhanovsky (1965) and Lafer (1992a).

Remarks: To date, 8 species of Endomychidae comprising 6 genera are known from Georgia (Tomaszewska 2007; Tarkhnishvili et al. 2013). The genus *Lycoperdina* includes 18 described species. They are usually found in an open place with scarce vegetation and develop in mushrooms of the Agaricaceae family, including species of the genera *Lycoperdon* P. Micheli, 1729; *Bovista* Persoon, 1794; *Handkea* Kreisel, 1989; *Calvatia* (Fries, 1849); *Mycenastrum* (Desvaux, 1842); *Tulostoma* Persoon, 1794; and *Agaricus* Linnaeus, 1753 (Kryzhanovsky 1965; Lafer 1992a; Nikitsky et al. 1996; Wojas 2016; Lund 2017). *L. succincta* has a wide distribution range in Europe and Central Asia and was reported in Georgia from a single location – Mt. Lomis Mta (Radde 1899). This record was, however, overlooked by Tomaszewska (2007). Later, this species was also found in Lagodekhi by Japoshvili et al. (2022). Collecting the species from the bank of the Paravani River mouth at Saghamo Lake is the third finding and enlarges its known distribution eastward in Georgia.

Figure 3. *Lycoperdina succincta* (Linnaeus, 1767), Saghamo Lake. Scale bar = 1 mm.

Figure 4. *Pocadius adustus* Reitter, 1888, Nedzvi Managed Reserve. A – dorsal view; B – ventral view. Scale bar = 1 mm.

Figure 5. *Necrobia violacea* (Linnaeus, 1758), Saghamo Lake. Scale bar = 1 mm.

Family Nitidulidae Latreille, 1802
Genus Pocadius Erichson, 1843

Pocadius adustus Reitter, 1888

Materials Examined: GEORGIA • Nedzvi Managed Reserve; N41.9024°, E43.5161°; 885 m a.s.l.; 08.10.2021; ISU, CaBOL IDs: 1018762, 1018763 (Fig. 4); leg: CaBOL team.

Genetics: The BOLD identification engine indicated high similarity of the sequence yielded from the specimen with CaBOL ID 1018763 (BOLD: ACJ7127) to the COI of *Pocadius adustus* from Germany (BIN ID: ACJ7127, similarity 98.95%). The morphology of the Georgian specimens also perfectly corresponds to the description provided in the key by Medvedev (1965).

Remarks: To date, 63 species of Nitidulidae comprising 23 genera are known from Georgia (Jelinek and Audisio 2007; Tarkhnishvili et al. 2013). The genus *Pocadius* includes 10 described species, displaying peculiar habits of being epigeous gasteromycetes specialists (Cline 2008). *P. adustus* has a wide distribution range in the Palaearctic region, with the nearest findings from Turkey (Jelinek and Audisio 2007) and the Western Caucasus in Russia (Cline 2005). The specimens from the Nedzvi Managed Reserve found in the puffball (*Lycoperdon* sp.) cavity represent a first record for Georgia.

Family Cleridae Latreille, 1802
Genus Necrobia Latrielle, 1797

Necrobia violacea (Linnaeus, 1758)

Materials Examined: GEORGIA • Saghamo Lake; N41.2941°, E43.7309°; 2006 m a.s.l.; 11.10.2021; ISU, CaBOL ID: 1018769 (Fig. 5); leg: CaBOLD team;

Genetics: The BOLD Identification Engine indicated high similarity of the sequence yielded from the specimen with CaBOL ID 1018769 (BOLD: AAX0338) to the COI of *Necrobia violacea* from Germany (BIN ID: AAX0338, identity 99.85%).

Remarks: To date, 16 species of Cleridae comprising 10 genera are known from Georgia (Tarkhnishvili et al. 2013). Of the 14 species reported from Georgia by Zaitsev (1915) in his attempt to review the fauna of checkered beetles of the Caucasus on the basis of literature, his own collections, and the material stored in the collection of the Caucasian Museum (Currently the Simon Janashia Museum of Georgia), he also reported *N. violacea* from Georgia. However, from this work, 4 species – *N. violacea*, *N. rufipes* (Fabricius, 1781), *Korynetes caeruleus* (De Geer, 1775) and *Tillus elongatus* (Linnaeus, 1758), have been overlooked by subsequent authors in the respective works (Richter 1965; Krivolutskaya 1992; Löbl and Rolcik 2007), only indicating the "Caucasus" without specifying the exact location.

Figure 6. *Byrrhus pustulatus* (Forster, 1770), 2 km SW of Ijareti. Scale bar = 1 mm.

The cosmopolitan *N. violacea* has a wide distribution range in the Palearctic region. It was reported from Georgia from Telavi, Tbilisi, Mtskheta, and Tana (Ateni Gorge) (Zaitzev 1915). Although it is a predatory beetle, it shows necrobiontic behavior and often can be found on animal carcasses (Krivolutskaya 1992). The morphology of the specimen from the bank of the Paravani River mouth at Saghamo Lake, collected from a cow skull, perfectly corresponds to the description provided in the keys by Richter (1965) and Krivolutskaya (1992). This new locality represents significant range expansion in Georgia.

Family Byrrhidae Latreille, 1804

Genus Byrrhus Linnaeus, 1767

Byrrhus pustulatus (Forster, 1771)

Materials Examined: GEORGIA • South West of Ijareti; N41.6490°, E42.7012°; 1503m a.s.l.; 09.10.2021; ISU, CaBOL ID: 1018729 (Fig. 7); leg: CaBOL team.

Genetics: The BOLD identification engine indicated high similarity of the sequence yielded from the specimen with CaBOL ID 1018729 (BOLD: ACL0075) to the COI of *Byrrhus pustulatus* from Germany (BIN ID: ACL0075, similarity 99.08%). The morphology of the Georgian specimen perfectly corresponds to the description provided in the keys by Arnoldi (1965) and Lafer (1992b).

Remarks: To date, 7 species of Byrrhidae comprising 4 genera are known from Georgia (Tarkhnishvili et al. 2013). The genus *Byrrhus* includes 31 described species, with both larvae and adults feeding on moss, algae, and liverworts (Lafer 1992b). So far, 4 species from the genus are known from Georgia (Jager and Putz 2016). *B. pustulatus* has a wide distribution in the Palaearctic region, with the nearest findings in Armenia and Azerbaijan (Jager and Putz 2016). The specimen from Ijareti represents the first record of the species from Georgia.

Conclusions

In the present study, we provide data on the distribution of little-known and understudied beetle species in Georgia, including two new records for the country. Although, in the past, significant attempts have been made to study the beetles of Georgia, knowledge is still strongly limited. This is shown by the regular discovery of new species and records in Georgia given the relatively low intensity of field studies (e.g., Lyubarsky et al. 2016; Aslan et al. 2017; Chaladze et al. 2017; Maghradze et al. 2019; Japarashvili 2021, 2022; Japoshvili et al. 2022). It is worth noting that the inaccessibility or lack of data from old and especially local, hard-to-reach, and little-known sources leads to an incomplete picture not only of the fauna of Georgia but the Caucasus region overall. In turn, this makes it difficult to correctly understand the diversity and distribution of beetles in Georgia and plan a conservation strategy. Retrieving the actual range within Georgia of the species listed above (and not only these) requires further studies that, in parallel, can help shed light on other, yet-undiscovered taxa from other poorly studied families. Thus, we encourage all interested coleopterologists and citizen scientists to intensify the research of Georgia's beetle fauna in order to support the species and community conservation in the long run.

Acknowledgments

The study was funded by the Federal Ministry of Education and Research under grant number 01DK20014A. The responsibility for the content of this publication lies with the author. Our special thanks to the Agency of Protected Areas for giving us the collection permit #655-0-2-202103182033.

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